

CLAIMS

I claim:

1. A method of purifying proteases, comprising the steps of
 - 5 a. constructing a protein expression vector which comprises a coding sequence for said proteases, a coding sequence for a protease autocatalytic site, and a coding sequence for an affinity chromatography binding site;
 - b. introducing said vector into a protein expression system compatible with said vector to produce an expression product comprising said
10 protease, said protease autocatalytic site, and said affinity chromatography binding site;
 - c. applying said expression product to an affinity chromatography substance;
 - d. removing said expression product from said affinity
15 chromatography substance; and
 - e. removing said protease from said proteases autocatalytic site and said affinity chromatography binding site through an autocatalytic process.
2. The method of claim 1, wherein said affinity chromatography binding
20 site comprises a polyhistidine.

3. The method of claim 2, wherein said polyhistidine comprises six histidines.

4. The method of claim 1, wherein said protease autocatalytic site
5 comprises at least one tripeptide selected from the group consisting of Phe-Leu-Arg, Phe-Val-Arg, Phe-Pro-Arg, Pro-Phe-Arg, and Leu-Phe-Arg.

5. The method of claim 1, wherein said proteases autocatalytic site is located between said protease and said affinity chromatography binding site.

10

6. The method of claim 1, wherein said proteases are kallikrein-like proteases from snake venoms.

15

7. The method of claim 1, further comprising the step of refolding said protease after step d.

8. A vector for expressing a protease capable of removing said proteases from an expression product through an autocatalytic process comprising the cleavage sequence selected from the group comprising SEQ ID 2 to 6.

20

9. The vector according to claim 8 wherein the SEQ ID is 2.

10. The vector according to claim 8 wherein the SEQ ID is 3.

11. The vector according to claim 8 wherein the SEQ ID is 4.

5 12. The vector according to claim 8 wherein the SEQ ID is 5.

13. The vector according to claim 8 wherein the SEQ ID is 6.

14. A group of purified protease made by the method of claim 1.

10

15. The purified proteases of claim 14, wherein the proteases are from
Trimeresurus mucrosquamatus.

16. A method of treating a cardiovascular disorder in an animal in need
15 thereof, comprising administering an effective amount of purified proteases of claim
14.

17. The method of claim 16 wherein the cardiovascular disorder is
hypertension.

20

18. The method of claim 16 wherein the cardiovascular disorder is a stroke.

19. The method of claim 16 wherein the cardiovascular disorder is thrombosis.

20. The method of claim 16, wherein the purified proteases are capable of
5 cleaving angiotensin I.

21. The method of claim 16, wherein the purified proteases capable of releasing bradykinin from plasma kininogen.

10 22. The method of claim 16, wherein said protease is capable of digesting N-benzoyl-Pro-Phe-Arg-p-nitroanilide.

23. The method of claim 16 wherein the purified proteases are a group of kallikrein-like proteins including Tm-VIG and Tm-IIG.